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Journal of Chromatography A, 785 (1997) 289–312

JOURNAL OF CHROMATOGRAPHY A

Review

Supercritical fluid extraction of pesticides in foods

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Abstract

This article summarizes research findings involving the supercritical fluid extraction (SFE) of pesticides in food and other tissue matrices. Emphasis is placed on multiresidue analysis of pesticides in nonfatty foods, including some previously unpublished aspects of SFE in this application. Brief overviews of pesticides and traditional multiresidue methods are given, followed by discussion of results for SFE applications in the pesticide residue analysis of foods. © 1997 Elsevier Science B.V.

Keywords: Supercritical fluid extraction; Multiresidue analysis; Reviews; Pesticides

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0021-9673/97/$17.00
PII S0021-9673(97)00461-5

1. Introduction

Before the development of commercial supercritical fluid extraction (SFE) instruments in the late 1980s, few researchers investigated the use of supercritical fluids in analytical, nonchromatographic applications. Increased automation and improvements in instrument design created a surge in SFE research in a variety of applications during the early 1990s. SFE has been the subject of many books and congresses [1–17], international conferences, general/fundamental reviews [18–32], and since 1988, a journal (J. Supercrit. Fluids) has been dedicated to studies involving supercritical fluid technologies. SFE has grown to the extent that SFE reviews often concern specific applications, such as environmental contaminants [33–42], fats and oils [43–46], natural products [47–51], industrial applications [52–54], metals and ions [55–57], and others [58–62]. However, only two previous articles have specifically reviewed the use of SFE in pesticide analysis [63,64].

The advantages of SFE are well-known by now, however, the limitations of SFE have not been as widely advertised. The advantages of savings in labor, operational costs, laboratory space, waste minimization, and increased selectivity with SFE versus traditional methods are generally true. However, practical matters taken for granted or solved long ago with traditional approaches (such as sampling protocols, availability of specialized support equipment and materials, and certain arbitrary criteria for method acceptance) must be re-addressed if SFE is to replace or supplement traditional methods on a large scale. Also, commercial instrument design and performance has not yet reached its full potential in sample throughput, affordability, and reliability. Furthermore, the general lack of experience and time required for SFE method development have made implementation of SFE in routine analytical applications a slow process. Inherent difficulties related to an incomplete theoretical understanding of SFE in complex applications, matrix effects on extraction, and the large number of variables to control have also compounded the situation. These issues and others will be addressed in this article.

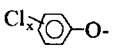
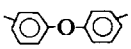
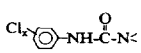
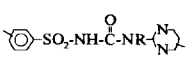
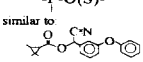
In light of the recent excellent review involving SFE applications for pesticides [64], a different format will be used for this article, and not all SFE studies involving pesticides will be referenced. This article is intended for analytical chemists who regularly analyze pesticides, but do not currently use SFE, and for established users of SFE, who do not work with pesticides. A brief overview of pesticides is provided for those who may wish to compare pesticide and matrix interactions with analytes and matrices encountered in other SFE applications. SFE of pesticides in soil, water, and air have been the subject of previous SFE reviews [33–42,64], and further discussion of these studies would be redundant.

2. Pesticides

As defined by the US Environmental Protection Agency, the term 'pesticide' is often used to designate 'any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest'. These pests can be divided into categories such as weeds, insects, fungi, mites, slugs, nematodes, rodents, and micro-organisms, which form the basis for the more specific terms, 'herbicide,' 'insecticide,' 'fungicide,' 'acaricide,' 'molluscicide,' etc. In the past, pesticides have been referred to as 'agrochemicals,' and more recently, 'pest control agents' or 'compounds of agricultural significance' but for the purpose of this article, the more specific terms will be used.

Table 1 provides a very abbreviated synopsis of major families, or chemical classes, of pesticides grouped by their uses. In analytical applications, an important feature in extraction is the polarity of the analyte, and the solubility ranges of the different pesticide families in water (a practical measure of the polarity of a chemical) are presented in Table 1. The basic structure of a pesticide within a family is also presented, but of course, the various groups attached to the basic structure affect analytical aspects of a pesticide as well. Unfortunately, the structures and properties of each pesticide cannot be presented in this article. For more information, pesticide handbooks [65–69], including a convenient electronic version of the *Farm Chemicals Handbook* [68] are available. The Pesticide Properties Database [69],

Table 1
Major types of pesticides

Use: family	Solubility Range in Water (mg/L)	Examples	Basic Structure
Fungicides:			
dithiocarbamate	10^{-1}-10^{5}	mancozeb, maneb metiram, zineb	
imidazole	10^{0}-10^{4}	carbendazim, imazalil, thiabendazole	
phthalimide	10^{0}	procymidone, captan, captafol, folpet	
triazole	10^{1}-10^{3}	myclobutanil, propiconazole	
Herbicides:			
acetamide	10^{2}-10^{4}	alachlor, dichormid, metolachlor	
chlorophenoxy	10^{1}-10^{6}	2,4-D, 2,4,5-T, MCPA, silvex	
dinitroaniline	10^{-1}-10^{0}	pendimethalin, trifluralin, dinitramine	
imidazolinone	10^{1}-10^{6}	imazaquin, imazapyr, imazethapyr	
phenylphenoxy	10^{-1}-10^{6}	fomesafen, bifenox, fluazifop-butyl	
phenylurea	10^{0}-10^{3}	linuron, diuron, thidazuron, neburon	
sulfonylurea	10^{2}-10^{4}	chlorsulfuron, chlorimuron-ethyl	
thiocarbamate	10^{0}-10^{6}	vernolate, asulam, butylate, thiobencarb	
triazine	10^{0}-10^{4}	atrazine, ametryn, simazine, prometon	
Insecticides:			
carbamate	10^{1}-10^{5}	carbofuran, aldicarb, propoxur, oxamyl	
organochlorine	10^{-3}-10^{0}	methoxychlor, DDE, lindane, endosulfan	insecticides containing chlorine
organophosphorus	10^{-1}-10^{6}	diazinon, chlorpyrifos, acephate, ethion	
pyrethroid	10^{-3}-10^{-1}	permethrin, cyfluthrin, fenvalerate, bifenthrin	

which can be accessed by internet (http://ncsr.arsusda.gov), is also a good source of information. The FDA (http://vm.cfsan.fda.gov/list.html) and several other organizations, as linked by Virginia Tech University (http://www.vtpp.ext.vt.edu/htmldocs/sitelist.html), maintain other sources of information about pesticides on the internet. The wide variety of physicochemical properties and chemical structures of pesticides make them an interesting set of analytes for investigations. The many possible matrices (e.g., formulations, plant and animal tissues, textiles, water, soil, and air), and trace concentrations at which residues may appear, often make analysis of pesticides a challenging task.

Fig. 1 sorts the 340 pesticides in the Pesticide Properties Database by solubility in water. This figure is not a completely accurate representation for all pesticides most commonly found in food and the environment (those issues are determined by farming practices, region, commodities, weather, time of year, and a host of other factors), but it represents a good cross-section of common pesticides. Herbicides are by far the most commonly used pesticides, followed by insecticides, fungicides, and others [70].

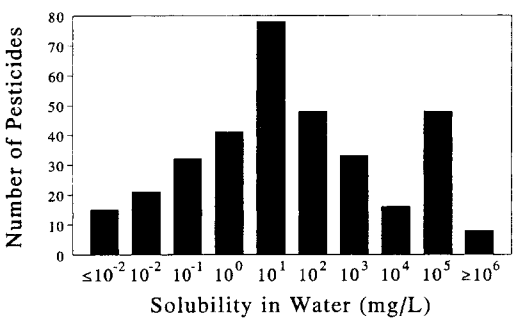


Fig. 1. Range of pesticide solubilities in water for the 340 pesticides in the Pesticide Properties Database [69].

As shown in Fig. 1, most of the pesticides have solubilities in water from 10–100 mg/l at 20–25°C, and another peak appears at higher solubilities due to the number of pesticides that are listed as salts. The coincidence that SFE using CO_2 is well-suited for extraction of chemicals in that solubility range means that most pesticides can be extracted without difficulty [71]. The most polar and nonpolar pesticides, however, may require the use of particular techniques (addition of solvent modifier, salt, ion-pairing or derivatization agent) as will be discussed in following sections.

3. Traditional multiresidue methods

In general, the most efficient way for a laboratory to save time, labor, and cost is to use the fewest possible methods for analysis of the most analytes. However, the inherent problem with a multiresidue approach is that matrix co-extractives tend to increase as the method includes a wider polarity range of analytes. No current method can extract all pesticides from all matrices with a single approach. Method development chemists for multiresidue analysis must resolve two defining questions: (1) What should be the polarity range for a method to recover the maximum number of analytes with the fewest matrix interferants? (2) To what extent should recovery be sacrificed to increase selectivity, or vice versa?

In regulatory analysis of pesticides, the answers to these questions are framed by: (1) health and environmental safety concerns that prioritize which pesticides must be monitored; and (2) minimum analytical criteria for results which are established by quality assurance protocols. Beyond these two constraints, the answers to the questions should be answered by minimizing cost and maximizing convenience of an overall scheme of methods for the pesticides and matrices of interest with available analytical instrumentation.

In many cases, a degree of accuracy can be sacrificed to achieve high sample throughput with minimal cost and effort, but other times, such as legal cases, or to assess exposure and risks, accuracy is more important. In the regulatory analysis of foods, factors external to the analysis (sample perishability, personnel, budgets) often form additional constraints of how much time and effort is allotted per sample. For purposes of food safety, screening methods are often used to increase the number of samples that can be analyzed. Ideally, fast and accurate analytical methods for a wide range of analytes can be developed to achieve the goals of both purposes.

Traditionally, organic solvents have been used for extraction of pesticides. With liquid solvents, selectivity is controlled by the choice of solvent, with hexane forming one of the most nonpolar solvents, and methanol and water at the polar end of the solvent spectrum. Solvents are often combined, as in HPLC, to achieve a desirable viscosity and solvating strength appropriate to the extraction. A liquid is an excellent medium to homogenize a sample, limit degradation and volatilization processes, and conveniently allow the use of salts, acids and bases, and partitioning techniques. High selectivity can be obtained using liquid-based extraction and clean-up via liquid–liquid partitioning and chromatographic means. However, these type of clean-up and the associated solvent concentration steps take time, labor, space, specialized equipment, and add to the expense. With sample size reduction, however, many aspects of liquid-based extraction can be automated, and the associated limitations with traditional approaches minimized [72].

Table 2 lists and compares different methods of analysis for multiple pesticide residues in nonfatty foods. The most generally used method of multiresidue analysis for pesticides in fruits and vegetables is the FDA PAM I 302 E1 [73], or the 'Luke

Table 2
Comparison of liquid-based multiresidue extraction methods and SFE

Parameter/Ref.	FDA [75][a]	CDFA [76][b]	Swedish [78]	Canadian [77][c]	Dutch [79]	SFE (for PDP)[d]
Sample Size	100 g	50 g	75 g	50 g	15 g	50 g (3 g)
Acetone	367 ml	2 ml		40 ml	30 ml	3 ml
MeCN		100 ml		115 ml		
EtOAc			225 ml			
CH_2Cl_2	300 ml	10 ml			34 ml	
Pet-ether	23 ml				30 ml	
Iso-octane					4.5 ml	
Toluene				5 ml	0.5 ml	
Cyclohexane			25 ml			
n-Hexane		20 ml				
Solvents, total	690 ml	132 ml	250 ml	160 ml	99 ml	60 g CO_2
Estimated time	3 h	2 h	2 h	2 h	1.5 h	1.5 h
Labor	manual	manual	manual automated GPC	manual	manual automated SPE	automated
Space	lab	lab	lab	lab	bench+hood	bench
Glassware	>10 items	>10 items	<5 items	<10 items	<5 items	jar, vial
Equipment	Blender SPE set-up Steam-bath Filters	Blender Steam-bath SPE set-up N_2 evap. Filters	Blender GPC system Rotovap N_2 evap.	Blender Centrifuge N_2 evap. SPE set-up Rotovap	Blender Centrifuge SPE set-up	Blender SFE
Chemicals	C-18 SPE Na_2SO_4, 5 g NH_2 SPE QMA SPE	NaCl, phosphate Na_2SO_4, 20 g C-18 SPE NH_2 SPE	Na_2SO_4, 60 g SX-3 GPC column, 40×1 cm	NaCl, 10 g Na_2SO_4, 10 g NH_2 SPE	NH_2 SPE	Hydromatrix, 50 g $MgSO_4$, 3 g ODS trap
Material cost[e]	≈$13	≈$10	≈$5	≈$9	≈$2	≈$3

[a] Many modifications of FDA methods are advantageous to the one presented here.
[b] CDFA modification of published approach is to use less hexane in azeotrope to speed MeCN evaporation.
[c] Canadian method modified to use NH_2 SPE clean-up cartridges rather than published celite:charcoal approach.
[d] Not necessarily the most advantageous SFE method.
[e] Costs vary depending on vendors, but they are taken from the same vendor in this table—other costs not presented include labor, instrumentation and indirect charges.

procedure' [74]. Table 2 presents aspects of a published modification of the method, or 'Luke II,' which was used for GC–ion-trap detection (ITD) analysis [75].

Due to the same factors that have been used to market SFE as an alternate method, regulatory laboratories around the world have been developing and implementing less costly methods. In 1991, Lee et al. published the method used by the California Department of Food and Agriculture (CDFA) [76]. In Canada, Fillion et al. also used acetonitrile (MeCN) for extraction [77]. More recently, the Canadian Pest Management Regulatory Agency modified their procedure to rely on 3 commercial solid-phase extraction cartridges for clean-up, which substantially increased the material cost per analysis. The Swedish multiresidue method uses ethyl acetate (EtOAc), which has some advantages for certain pesticides versus acetone [78], and is amenable for GPC clean-up. Acetone is still the most commonly used extraction solvent, mainly because of its price, low toxicity, and when combined with ≈30% water from the sample, it forms a strong solvent for a wide range of pesticides [73]. However, much clean-up is often required before analysis.

Often, the easiest and most effective way to reduce matrix background is to reduce sample size, and de Kok et al. of The Netherlands General Inspectorate for Health Protection developed a modified Luke procedure for a 15 g sample [79]. The Dutch method uses only one clean-up step, a liquid–liquid partitioning of the extract with petroleum

ether–dichloromethane, which is conveniently and rapidly performed in the same blender jar as the sample extraction. After centrifugation and a solvent concentration/exchange step, the extract is ready for GC analysis. This method relies on GC–ITD technology in chemical ionization (with MeCN) and electron impact modes, as well as selective GC detectors for certain pesticides, to monitor >200 pesticides at subtolerance levels in the complex extracts [79]. All of the methods (except SFE) in Table 2 require SPE clean-up before HPLC analysis of carbamate insecticides and benzimidazole fungicides, which can be automated [80,81]. The injection of 'dirty' extracts in GC–ITD in the Dutch approach is directly opposite to the more extensive clean-up approach used by Cairns et al. [75]. The reason is that US EPA and FDA regulations require that quantitation standards be prepared in neat solvents, whereas standards prepared in blank matrix, as in the Dutch method, have been shown to give accurate results for less clean extracts [82].

Table 3
Parameters in SFE

Sample preparation for SFE
Comminution procedure
Sample size
Water content
Drying agent? (type, ratio)
Vessel volume
Packing density
Extraction
Extraction fluid
Temperature
Pressure
Modifier? (type, amount)
Static time
Dynamic time
Flow-rate
Trapping
Sorbent type or solvent
Trap size or solvent volume
Collection temperature
Trap elution solvent
Trap elution volume,
Temperature and flow-rate
Clean-up?
Analysis

4. Method development in SFE

SFE is an easily automated procedure that essentially obviates the use of hazardous solvents and reduces the time, cost, manual labor, glassware, and lab space required for extraction. The polarity range for simultaneous extraction of possible analytes by SFE cannot match the range possible using traditional liquid solvents using most commercial instruments. For that reason, some believe that SFE with CO_2 is not suitable for simultaneous extraction of a wide polarity range of analytes. Again, the defining question mentioned for multiresidue methods must be addressed: What is the most efficient way to meet the needs of the analysis? SFE typically achieves a higher degree of selectivity during the extraction process, unlike liquid-based extractions, which generally use less convenient post-extraction clean-up steps to gain selectivity.

Table 3 lists the major parameters to be controlled in SFE method development studies. Others have stated their opinion in how to develop an SFE method [27]. In this author's opinion, the chemist should work systematically upward through each parameter listed in Table 3. One parameter may have an effect on a previously tested parameter which may require repetitive experiments. The first step is to ensure that the analytical method is reliable and consistent for the analytes of interest. Then, each trapping parameter should be addressed individually by spiking the sorbent trap (or collection solvent) and blank matrix in SFE. When trapping and elution efficiencies have been determined, the effects of extraction parameters should be determined in an incurred matrix, if possible. A good way to ensure complete extraction is to re-extract the vessel contents after SFE with a traditional method. Replicates, quality controls (blanks to ensure no carry-over), and instrument maintenance must be performed to ensure validity of findings.

4.1. Sample preparation

A fundamental parameter not often considered in SFE method development studies involves sampling protocols for SFE. The primary concern in developing a method is to determine how small of a subsample can be taken to (1) achieve a reproducibly representative sample, and (2) obtain the required

detection limit for analysis. In SFE, the sample must often be homogenized without the aid of a liquid, thus, a higher potential of analyte degradation and volatilization results. Furthermore, the absence or presence of water in the sample plays a very important role in SFE.

4.2. Sample size and homogeneity

In dry samples, such as grains and sand, homogenization of samples is not difficult, but with moist and heterogeneous samples, such as meats and produce, comminution becomes a very important process for accurate subsampling. Surprisingly, this issue has not been addressed extensively in the analytical literature. In regulatory analysis of foods, the FDA requires that a 20 lb sample be comminuted with a vertical cutter, and a 100 g representative sample be taken for analysis [73]. Recently, Young et al. showed the effect of taking smaller subsamples for methoxychlor in apples, cabbage, and green beans [83]. Previously, Hemingway et al. showed the effect of sample size reduction for cypermethrin in cabbage and apple [84]. Di Muccio et al. showed that condition of the sample and type of cutting equipment used were important factors in what sample size was representative [85]. The conclusions of this study was that comminution of frozen samples gave better homogeneity than for unfrozen samples, and that the use of a Robot Coupe chopper gave a more homogeneous sample than 2 other types of cutters. Lehotay et al. performed a study to determine sample homogeneity of chlorpropham, hexachlorobenzene, and lindane in potato for subsampling in SFE [86]. This and other studies have shown the advantages of mixing frozen samples [86–89]. The use of dry ice or liquid nitrogen can be a way to rapidly freeze a sample (a convenient way to make dry ice, of which many laboratory personnel are not aware, is to attach a hose to a cylinder of CO_2 and open the valve). Based on the results of the studies listed above, the consistent statistically representative sample size for produce was >10 g, but in some cases, <10 g subsamples were statistically representative.

Currently, maximum vessel size for commercial automated SFE instruments is 7–10 ml, which can contain 1–8 g samples, depending on the sample. Using traditional sample preparation methods, this range of subsample size is generally too small in fruit and vegetable applications to accurately represent the larger sample (further investigations may disprove this statement). However, Lehotay et al. demonstrated that, for potato, a typical laboratory blender can be used to prepare a homogeneous mixture of a larger, representative sample with Hydromatrix, which can then be subsampled for SFE and maintain high analytical precision and accuracy [86]. Further study of this critical component must be verified if SFE and other methods using reduced sample size are to be implemented.

Other ways to solve this sample homogeneity problem have also been used. Nelson and Abdelmesseh decided to forego the sample minimization procedure due to FDA sample size requirements and took a 1 ml portion of a 100 g sample extracted with 200 ml acetone [90]. SFE was being investigated to replace the liquid-based clean-up steps. They added the 1 ml portion to $\approx$7 g Na_2SO_4 in a 5 ml sample vessel. Extraction was at 200 atm and 75°C (0.63 g/ml) for 20 min with a Suprex Model SFE/50 (50 μm I.D. capillary restrictor), and collection was in 2.5 ml acetone. Recoveries for methamidophos, chlorpyrifos, acephate, dimethoate, monocrotophos, captan, chlorothalonil, vinclozolin were all $>80\%$ with this approach. Also, incurred residues of endosulfans, diazinon, and malathion were determined in SFE experiments (note that the use of this technique with collection on a sorbent rather than bubbling into solvent, requires a higher trap temperature than the boiling point of acetone or else the 1 ml acetone will be detrimental to trapping efficiency [91]). Detection limits with this approach were $\approx$20 times higher than with the traditional approach due to the small portion of the acetone extract taken.

Argauer et al. used a similar approach for meat samples, and to increase sample size, they concentrated the MeCN extract onto Hydromatrix [92]. Others have used a solid-phase sorbent for analyte concentration and isolation, which also obtains a representative sample for SFE [93,94]. Instead of evaporating the solvent onto the sorbent as in the above approach, the sample or extract is concentrated by flowing the liquid through the sorbent. The sorbent is then placed in the vessel and analytes are eluted with the supercritical fluid. This type of approach loses time-saving advantages of SFE and in

effect, SFE becomes a clean-up step in a liquid-based extraction method. Usually, only small amounts of organic solvents are needed for rapid and convenient elution in SPE, but there may be instances when supercritical fluid provides more selective elution of analytes than a liquid.

Water removal via oven drying or lyophilization is another option for reducing the volume of a wet, representative sample so that it may fit in an SFE vessel [95,96]. Fruits and vegetables are 80–95% water [74], and large gains in sample size for SFE can be made by simply removing the water. This approach is not recommended for many pesticides due to: (1) analyte volatilization, (2) additional time and equipment involved, (3) reduced extraction efficiency of many analytes in a dry matrix, and (4) the increased concentration of matrix components that may overwhelm the trapping system or create the need for additional clean-up of the extract [97,98]. However, complete or partial removal of water may be a good approach for extraction of nonvolatile pesticides from some matrices.

Another option to better ensure representative sampling in SFE is to simply increase sample size. Manually operated analytical SFE instruments are commercially available with up to 100 ml vessels. Unlike traditional approaches, in which time for extraction is not affected much by sample size, the time and operational costs of extraction increases proportionally with sample size in SFE at a given dynamic flow-rate. King and his collaborators avoided some of these disadvantages by designing and manufacturing their own SFE instruments [99,100]. Advantages of these instruments with respect to commercial instruments include: (1) up to 6 samples are extracted in parallel rather than sequentially which increases sample throughput; (2) vessel sizes are 154 ml and 98 ml which allows use of larger samples; (3) the instruments are capable of high flow-rate; and (4) they use inexpensive, commercial-grade liquid CO_2, which undergoes clean-up with an in-line column containing 24 g coconut charcoal and 48 g alumina C [100]. This type of instrument would be very expensive commercially, and reducing sample size saves costs. Ultimately, as with any method, the sample size chosen for extraction should be the smallest possible that is representative and permits detection of analytes at the desired concentrations.

4.3. Effect of water in SFE

Water in the sample often causes strong effects in SFE. There have been applications of direct SFE of aqueous samples [62], but precautions must be taken to avoid damage to the instrument. In effect, for samples with high water content, the water must be removed or controlled before performing SFE. Water can aid in the extraction process, or be detrimental, depending on water content, the analyte, and the matrix. Water can open pores and swell the matrix to allow the solvent better access to analytes of any polarity and aid flow through the matrix [101]. Also, even though water is only ≈0.3% soluble in supercritical CO_2 [102], it serves to increase the polarity of the fluid and enable higher recoveries of relatively polar species. However, if excess water remains in the vessel, a highly water soluble analyte will prefer to partition into the aqueous phase and its SFE recovery will be low. Semi-polar analytes will be dissolved in the aqueous phase, but readily partition into the supercritical CO_2, and yield high recoveries. For analytes that are insoluble in water, the analytes precipitate onto matrix surfaces, and even though the analyte may be very soluble in the extraction fluid, the excess water in the sample acts as a barrier in transfer of the analyte to the fluid.

Fig. 2, which was prepared from data reported by Lehotay and Lee [71], shows the effect of water on SFE of pesticides of different solubilities in water. In the paper, they show the difference between a dry and wet matrix for a wide range of pesticide

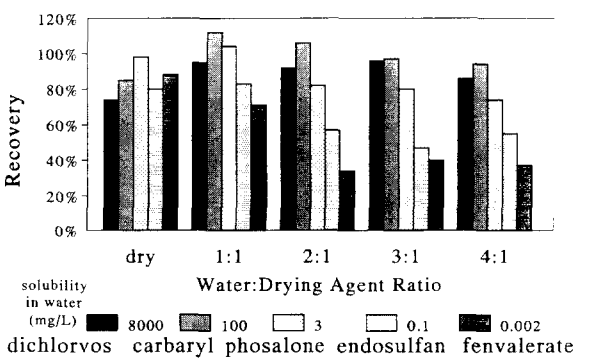


Fig. 2. The effect of moisture in SFE using a fibrous cellulose powder, CF-1, as drying agent for pesticides of different solubilities in water.

polarities. Nemoto et al. show further evidence of this effect in their controlled study of modifiers and SFE instrument parameters [103]. For soil samples, Snyder et al. demonstrate the effect of water in a thorough study of all extraction parameters [104,105].

4.4. Drying agents

If water is not removed from a sample, it must be controlled in SFE to keep it from affecting the restrictor, trapping process, or analysis. The use of drying agents is the most common way to retain water in SFE, and due to the dispersion of analytes in the matrix, it can also help in the extraction and sample homogenization processes. The properties of an ideal drying agent for SFE consist of: (1) high absorptivity of water, (2) good sample consistency, (3) low cost, (4) no heating upon hydration, (5) inert, (6) cause no analytical interferences, and (7) pose no hazards to health or the environment. Table 4 lists some drying agents and their features that have been tested in SFE. Burford et al. compared different drying agents in an environmental SFE application [106]. Hopper and King were the first to use Hydromatrix (HMX), a pelletized diatomaceous earth material, to absorb water for SFE [107]. They demonstrated its applicability in a 2:1 sample–HMX ratio to improve SFE recoveries for 34 pesticides in butter fat, meat, peanut butter, potato, carrot, and lettuce. Lehotay and Eller further demonstrated the utility of HMX for SFE of pesticide residues [108]. They determined that a 1:1 sample–HMX ratio was more applicable for SFE with their approach using commercial instruments for 46 pesticides in fruit and vegetable samples. Recently, Lehotay and Lee tested a fibrous cellulose powder, CF-1, as a drying agent in SFE [71]. Despite the high water absorptivity of the drying agent, the consistency of the matrix was too fluffy to allow good sample homogeneity for the mixture. Also, CF-1 exhibited a similar tendency as celite and HMX to retain the phosphoramide pesticides, methamidophos, acephate, and omethoate.

Oostdyk et al. offered a likely explanation of this retention behavior [109]. The hydroxyl groups present in the matrices form hydrogen bonds with the amine and, to a lesser extent, phosphate groups on the pesticides. Valverde et al. demonstrated that the use of $MgSO_4$ as a drying agent for peppers and other commodities in SFE enabled high recoveries of methamidophos and other pesticides [110]. It is likely that the $MgSO_4$ also serves to desalt the highly water soluble phosphoramide pesticides from the water phase in SFE to further increase their recoveries. In a subsequent study, they showed that SFE with $MgSO_4$ drying agent was applicable for other pesticides, but not imidacloprid [111].

$MgSO_4$ has disadvantages as a drying agent versus HMX in that it is more expensive, heats upon hydration, consists of fine particles that can harm the restrictor, and forms rocks in sample mixture. Eller and Lehotay showed that a mixture of 2:2:1 sample–

Table 4
Comparison of selected drying agents for use in SFE

Drying agent	Saturation ratio (water:drying agent)	Cost (cents/g)	Heat of hydration	Density (g/ml)[c]	Consistency	Notes
Celite 545	3:1[a]	1.2	None	0.36	Compact	Retains phosphoramides
Hydromatrix	3:1[a]	3.8	None	0.29	Pelletized	Retains phosphoramides
Cellulose, CF-1	4:1[a]	5.0	None	0.21	Fluffy	Retains phosphoramides
Alumina	1:1[a]	2.4	None	0.85	Compact	Retains polar pesticides
Florisil	1.5:1[a]	9.5	None	0.50	Compact	Retains polar pesticides
Molecular sieves[d]	0.5:1[a]	4.0	Moderate	0.83	Variable	Unknown effects on pesticides
Magnesium sulfate	1.05:1[b]	5.0	High	0.62	Powdery	Hinders nonpolar pesticides
Sodium sulfate	1.27:1[b]	2.2	Low	1.4	Grainy	Hinders nonpolar pesticides

[a] Determined by addition of water at room temperature.
[b] Determined by calculation using molecular weights.
[c] Amount of dry material packed in an extraction vessel divided by volume.
[d] Molecular sieves 5A ground with mortar and pestle.

$MgSO_4$–HMX maintained advantages, and minimized disadvantages, of each drying agent. They obtained high recoveries of 75 pesticides including phosphoramides [112]. They postulate that the $MgSO_4$ interferes with hydrogen bonding interactions with the HMX, and also helps to desalt the highly water soluble pesticides out of the water phase in the sample and into the supercritical CO_2, thereby increasing their recovery in SFE. A further benefit of the use of salts is the destruction of microorganisms and enzyme activity that can degrade pesticides during storage of food samples awaiting SFE [86]. Stefani et al. developed a multiresidue SFE procedure using 2:2.5:0.8 sample–Na_2SO_4–celite which was shown to give high recoveries for 92 diverse pesticides [113]. Longer extraction times were required for the phosphoramides.

Fig. 3 shows this effect of salt for the semi-polar insecticide, malathion, and the polar phosphoramide, omethoate, in orange on HMX and a 2:2:1 mixture of apple–$MgSO_4$–HMX. Burford et al. also observed retention of nonpolar analytes on $MgSO_4$ [106], and Hopper described instances of retention of certain pesticides on Na_2SO_4 in classical extraction methods [114]. Pesticide recoveries reported by Stefani et al. also exhibit this trend for nonpolars [113]. The combination of salt and water in SFE has been shown to dramatically affect pesticide recoveries, and in comparison, altering instrumental extraction parameters have relatively minor consequences.

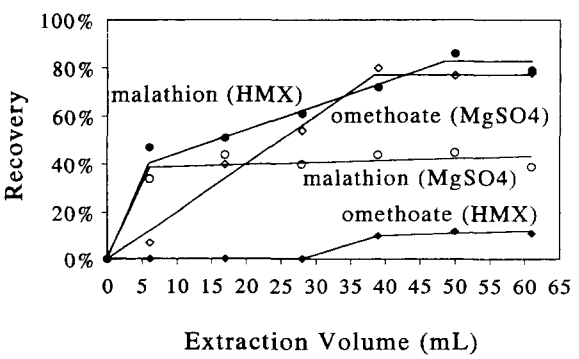


Fig. 3. Effect of $MgSO_4$ to improve SFE recovery of a phosphoramide pesticide, omethoate, and to reduce recovery of malathion, a less polar pesticide, from 2:2:1 orange–$MgSO_4$–HMX versus 1:1 orange–HMX.

This situation has forced the use of 2 extractions per sample to achieve high recoveries of both nonpolar and phosphoramide pesticides in a pilot study designed to implement SFE in the Pesticide Data Program. The first extraction uses 1:1 sample–HMX to extract the majority of pesticides, then $MgSO_4$ is added to a second subsample of the same mixture for SFE of methamidophos, acephate, and omethoate. Aspects of this procedure are listed in Table 2, and time and cost of analysis could be halved if a single method were used to accomplish the same range of pesticides. It should be noted that all multiresidue procedures have difficulties with certain pesticides [73].

4.5. Sample particle size and packing density

Other parameters often overlooked in SFE method development studies are sample particle size and packing density. In general, decreasing particle size in SFE, particularly in the case of natural products, creates more surface area and benefits extraction, but it also my hinder extraction if the analytes re-adsorb on matrix surfaces. Bøwadt and Hawthorne discuss elution of the analytes from the matrix in the vessel as a fundamental component in the extraction process [38]. To determine the rate limiting step in SFE, they discuss conducting an experiment to determine the effects of flow-rate and dynamic time on extraction. A higher flow-rate can help reduce partitioning back onto matrix sites if this is the limiting factor (otherwise, solubility factors are limiting). Also, larger particles, decreased packing density, smaller sample size, and a wider extraction vessel reduce this potential matrix effect. Sample particle size and vessel packing density uniformity in day-to-day operations may be a factor in some SFE applications that achieve variable results.

4.6. Modifiers

In a thorough study involving modifiers and instrumental parameters in SFE of 88 pesticides fortified onto celite, Nemoto et al. showed that water is the strongest modifier followed by methanol (MeOH) [103]. If a sample already contains water,

MeOH and other modifiers have a minimal effect. In theory, the miscible modifier may help increase the amount of water dissolved in the supercritical CO_2 and increase recoveries of all pesticides. Valverde et al. appeared to demonstrate this effect, and they chose to add 200 μl MeOH to their samples [110,111]. In other cases, modifiers gave no statistically significant improvement in pesticide recoveries, but neither did a 200 μl addition to the sample harm recoveries [115]. Use of higher amount of modifier has been shown to decrease recoveries [103]. Ashraf-Khorassani and Taylor showed that modifier addition to the sample tended to give higher recoveries than modifier addition with a pump during extraction [116]. Taylor's group also demonstrated the effect of solvent modifiers on trapping efficiency with solid-phase trapping in SFE [91]. Levy et al. also studied the effects of modifiers in pesticides, but their results were inconclusive [117].

In an example of using '100% modifier', Sundaram and Nott [118] compared extraction of aminocarb and fenitrothion from pine needles and soil using common extraction solvents (hexane, EtOAc, MeCN, and MeOH) at pressures and temperatures above and below the supercritical parameters. The critical pressures of the solvents were rather low (3–8.1 MPa, or <80 atm), but critical temperatures were >230°C. A homebuilt SFE instrument using an HPLC pump and GC oven was used for the study. The use of an extraction solvent that is a liquid at room temperature loses a key benefit of SFE with CO_2: concentration of the analyte(s) during trapping. The only advantage in the use of a liquid for SFE in this study was the solvent savings from 150 ml to 60 ml for an extraction. Commercial systems that also use heated, pressurized fluids (accelerated solvent extraction [71,119,120] and microwave assisted extraction [121]) do not operate above the critical temperatures and pressures of common solvents. Capriel and Khan observed a higher potential of breaking analyte–matrix interactions using supercritical MeOH [122], but this effect was not observed by Sundaram and Nott [118]. In fact, SFE recoveries were often lower than the recoveries obtained by mixing the sample with a liquid solvent in a blender. The study was interesting nonetheless due to several observations: (1) despite a smaller sample size and lower analyte recoveries, the SFE extracts with organic solvents were darker in color than the extracts from the traditional approach and gave more matrix interferants after clean-up; (2) the SFE procedure took 15 min (133%) longer than the liquid-based method; (3) increasing temperature had an adverse effect on pesticide recoveries (presumably due to lower fluid density or pesticide degradation); and (4) the order of solvent strength for extracting the pesticides under SFE conditions (MeOH>MeCN>EtOAc>hexane) was different from the order at room temperature and pressure (EtOAc=MeCN>MeOH>hexane). Blackwell et al. also observed changes in the order of solvent strengths in supercritical states [123], which partially explains how poor solvents for multiresidue extraction (water and MeOH) have strong modifier effects in SFE [103].

4.7. Extraction parameters

Once the pre-extraction/sample preparation problems have been solved, the development of SFE instrumental extraction parameters are usually not difficult. Factors to determine include temperature, pressure, static time, flow-rate, dynamic time (fluid volume), and % modifier during the extraction. Hawthorne's group and others have shown that high temperatures can increase recovery of nonpolar analytes in native environmental samples [38]. Some pesticides, such as captan, readily degrade at such high temperatures, and moderate temperatures should be used unless such temperatures are possible and necessary.

In SFE, pressure should be used as a parameter to control fluid density at a given temperature. The effect of higher temperatures to increase solubility is a basic tenet in chemistry known even by laypersons, but in most instances involving SFE, recoveries of analytes correlate with CO_2 density independent of temperature (because solubility is adequate at the temperatures studied). Assuming equal results, whether to use high temperature and low pressure, or high pressure and low temperature, is a matter of debate. In practice, moderate pressures and temperatures are more commonly used. In SFE application of rather polar veterinary drugs (sulfonamides) in food matrices, Parks and Maxwell, chose to use high pressure at moderate temperature to achieve a CO_2

fluid density approaching 1.2 g/ml [124]. Some commercial instruments cannot achieve this elevated CO_2 density, and Din et al. investigated the use of modifiers, ion-pairing agents, and other factors in an attempt to improve solphonamide recoveries at lower density [125].

The choice of pressure and temperature in SFE to affect selectivity is a main advantage over mixtures of liquid solvents, which cannot achieve such control (but pH cannot be used in SFE to the extent possible with aqueous solutions). Control of density in SFE has enabled unique applications to separate classes of pesticides from common matrix interferants that can plague traditional methods. Nemoto et al. show the effect of CO_2 density for 88 pesticides fortified on celite [103]. Pesticides were separated into groups based on the density required to achieve recoveries >80%, with nonpolar pesticides being extractable at density <0.5 g/ml, and polar pesticides requiring more than 0.9 g/ml for extraction. Unfortunately, the researchers were limited by the instrument and higher densities could not be investigated.

It is widely believed by analytical application chemists that if an analyte is very soluble in supercritical CO_2 at low density, that this solubility will increase or remain the same at higher density. In fact, solubility forms a peak in a plot with density [126], but in analytical applications, this trend is usually not observed due to the low concentration of analytes.

Static and dynamic times and flow-rate are also important considerations in SFE. In most pesticide applications, static time is just long enough to allow the sample to reach the temperature of extraction. For pesticides that are not as easily extracted, increasing static time and/or performing multiple static extractions may increase recoveries. However, if the pesticide is poorly extracted using a short static step, longer and repeated static steps will not dramatically increase recoveries by itself.

Dynamic time in SFE is essentially a measure of elution volume as determined by flow-rate. Many nonpolar and moderately polar pesticides in food require only 1–2 elution volumes versus vessel size independent of flow-rate. More polar pesticides, or those that interact with matrix, often require >4 vessel volumes of extraction fluid. To achieve the maximum advantages of SFE, the shortest dynamic time at the highest flow-rate should be used. Unfortunately, flow-rates that can be used confidently with acceptable instrument performance are sometimes limited in commercial instruments due to restrictor and trap designs.

4.8. Trapping

Trapping in SFE has been the subject of numerous articles and often is the source of poor recoveries in nonoptimized studies [127–131]. Collection in SFE can be performed with a solid-sorbent, a solid surface, or a liquid, and analytes can be trapped before depressurization or afterwards. Trapping with liquids is generally nonselective, and provides high trapping efficiencies except for the most volatile components. The same aspect is true to a greater extent for trapping on inert surfaces [108,131]. The use of a solid-phase trap with adsorbent properties, even at elevated temperatures has been shown to be very effective for trapping in SFE [91]. It is not recommended to use collection temperatures below 0°C when water is present in the sample due to potential problems with ice. Trapping on solid-sorbents also allows a higher degree of selectivity by using liquid solvents to separate analytes from co-extractives. However, just as in traditional clean-up approaches, not all pesticides will elute with the desired solvent and volume from the trap. Currently, commercial SFE instruments with solid-phase traps limit the ability to adequately perform solid-phase clean-up of extracts due to the lack of a waste pathway and/or small collection vials. Until these factors are corrected, the use of a strong solvent to remove the analytes from the solid-phase trap in a minimal volume is the approach that must be taken [131]. Some degree of selectivity is still achieved, but more thorough clean-up, if necessary, can be performed afterwards or through in-line methods [125,132,133].

Eckard and Taylor compared trapping capacity of different solid-phase traps in SFE of test compounds [127]. The study was for high concentrations not typical of analytical applications. They determined that Porapak-Q possessed the highest trapping capacity among the sorbents tested. Lehotay and Valverde compared 4 traps (ODS, diol, Tenax, and Porapak-Q) and 4 elution solvents (acetone, EtOAc, MeCN, and MeOH) for more than 50 pesticides in 3 commodities [131]. Recoveries, elution volumes, and

clean-up aspects were compared for the different combinations. They confirmed the general use of ODS with acetone elution, which has been used frequently by others, as the best choice. In their experience with Porapak-Q, more solvent was required to elute pesticides from the trap, and the sorbent was incompatible with MeOH for GC analysis.

5. Multiresidue SFE methods

Several studies have determined the effect of instrument-controlled SFE parameters on pesticides on celite [103], soils [95,104,105], grains [134–137], and produce [108,110,113,203]. Table 5 gives different SFE conditions for multiresidue methods of pesticides in food. In comparing the methods, a CO_2 density of 0.8–0.9 g/ml appears to be adequate for most pesticides. Extraction volumes vary from 1.3 to 7.2 vessel volumes, depending on the instrument, pesticides/matrices, and if fortified or incurred samples were used for optimization of the method. Other aspects related to instruments from different manufacturers, such as collection approaches, appear in the methods. King et al. showed that methods developed on one instrument can be 'translated' to another with minor modifications [138].

Before the introduction of HMX in SFE by Hopper and King, analysis of moist foods posed difficulties in SFE due to clogging of the restrictor [107]. Hopper and King demonstrated that pesticides could be extracted from a variety of wet materials using the 'extraction enhancer,' but they did not present information pertaining to development of the extraction method. Their extraction conditions were selected in an attempt to completely extract the pesticides as well as lipids, which necessitated clean-up of extracts before GC analysis with selective detectors. Subsequent reports by Hopper et al. have maintained this approach [100,107], mainly due to the diversity of the pesticides and food samples analyzed in the FDA Total Diet Study and FDA requirements. Due to the need for post-extraction clean-up, particularly for fatty samples, the approach is not as expedient as the researchers desire, and work continues to address this issue [139].

Hosoi et al. performed SFE with a DKK Model LSA-1E for 31 OP and OC pesticides in orange juice, chicken, beef, corn, and wheat [140]. Table 5 presents reported conditions for the method. Recoveries for the pesticides were >85% in studies of spiked samples, and in 29 comparisons versus a traditional extraction method, results were in excellent agreement even at concentrations below 1 ng/g. No clean-up was used for nonfatty samples, and Florisil clean-up was used for fatty samples.

Lehotay and Eller performed method development using 2 commercial SFE instruments (Hewlett-Packard Model 7680T and Suprex Autoprep-44) for multiclass pesticide residues in fruits and vegetables [108]. They were confronted with the same range of pesticides as the Total Diet Study, but produce is a more consistent type of sample than processed foods. Due to (1) the lack of lipid co-extractives, (2) use of smaller sample size, (3) more selective extraction conditions, and (4) use of GC–ITD, clean-up of SFE extracts in this study were not required. As discussed earlier, subsequent studies were designed to further optimize each aspect of the procedure [86,112,131] for the pesticides in the Pesticide Data Program (PDP) [141]. The method appearing in Tables 2 and 5 was presented to the PDP which is conducting a pilot study to compare results of the SFE method with results from traditional methods for incurred samples. The results from this pilot study will be presented in the future.

In a study designed to determine if SFE can be implemented in the Israel Pesticide Residue Laboratory, Aharonson et al. compared results of SFE analyses versus the traditional multiresidue approach for foods [142]. Their SFE conditions for an Isco Model 2-10, which are translated from an existing method [108], are presented in Table 5. Subsamples of 2 g, taken from a 500 g sample, were mixed with HMX and extracted in SFE. In spiked sample studies in HMX, tomato, and cucumber, recoveries of malathion, chlorpyrifos, endosulfan, atrazine, azinphos-methyl, pyrazophos, metalaxyl, triadimeton, triademenol, methidathion, prochloraz, dichlorvos, hexaconazole, buprofezin, and carbosulfan exceeded 80%. Methamidophos and similar pesticides were not recovered with the SFE approach using HMX alone, and a rapid screening method may be possible for those pesticides [143]. Comparison of SFE results versus the Luke method was made for incurred samples in 21 instances involving 12 pesticides in strawberry, cucumber, apple, grape, and

Table 5
Comparison of SFE methods for extraction of multiple pesticide residues in food

Ref.	Sample prep.	Extraction	Collection/clean-up	Notes
[100,107,134]	26 g (produce) or 50 g (meat)+ 13 g HMX in 73 or 154 ml vessel	680 atm and 80°C (d=0.95 g/ml), 20 min dynamic at 10 ml/min (1.3–3 vessel volumes)	Collection in empty boiling flask, traditional clean-up, GC analysis	34 OP and OC pesticides in meat, fat, produce, grain and other foods
[140]	1 g sample+HMX in 10 ml vessel	250 atm and 50°C (d=0.85 g/ml), 20 min dynamic	Bubble into hexane, florisil clean-up for fatty samples, GC analysis	31 OP and OC pesticides, 29 determinations; agreement for 10 pesticides in 5 matrices
[116]	50 g+50 g HMX frozen, 5 g in 7 ml or 6 g in 10 ml vessel (2.5 or 3 g sample)	350 atm and 50°C (d=0.9 g/ml), 2 min static, dynamic at 2 ml/min for 6 vessel volumes	Collect on 1 ml ODS at 15°C, elute with 1–1.5 ml acetone, GC–ITD analysis	Validated for 50+ pesticides in produce low recovery of methamidophos
[142]	Homogenize 500 g frozen sample, 2.2 g+1.8 g HMX in 10 ml vessel	320 atm and 60°C (d=0.85 g/ml), 22 min dynamic at 1.8 ml/min (4 vessel volumes)	Bubble into 9:1 acetone–isooctane no clean-up, GC analysis	24 determinations for 12 pesticides in 5 commodities versus traditional method
[110,111]	20 g sample+28 g $MgSO_4$; 8 g (3.3 g sample) in 10 ml vessel+0.2 ml MeOH	300 atm and 50°C (d=0.88 g/ml), 1 min static, 15 min dynamic at ≈1 ml/min (1.5 vessel volumes)	Bubble into 3 ml EtOAc, no clean up, GC and HPLC analysis	High recovery of methamidophos and others, poor for imidacloprid
[113]	2 g sample+2 g $MgSO_4$+1 g HMX	350 atm and 50°C (d=0.9 g/ml), 2 min static, dynamic at 2 ml/min for 6 vessel volumes	Collect on 1 ml ODS at 10°C, elute with 1.5 ml MeCN, GC–ITD analysis	Good recoveries of methamidophos and others
[135]	Grind and homogenize frozen sample, 6 g in 10 ml vessel+2 ml 4:1 MeCN–H_2O	340 atm and 60°C (d=0.87 g/ml), 10 min static, 25 min dynamic, 1.5 ml/min (3.75 vessel volumes)	Bubble into 7 ml MeCN at 4°C, -NH_2 SPE clean-up, GC and HPLC analyses	Validated and implemented for 41 pesticides in grains
[136]	2 or 8 g sample in 3 or 10 ml vessel	150–200 atm and 50°C (d=0.71–0.79 g/ml)	Bubble into EtOAc–cyclohexane, no clean-up	Excellent agreement for 17 pesticides in 9 grain samples

spice samples. Samples of GC–MS chromatograms were given showing the ability to confirm the presence of pesticides in the SFE extracts not possible to confirm in the extracts from the traditional method due to matrix interferants. It was curious that the SFE results were higher for the same sample in 16 of the 21 instances for incurred samples. Initial results in the ongoing SFE pilot study in the Pesticide Data Program have also shown higher SFE results for apple. In liver, Snyder et al. also showed higher SFE results for pesticides than with a traditional method [144].

Two possible explanations for this result will be discussed: (1) the results from the traditional approach are inaccurate; or (2) the SFE results are inaccurate. The former possibility will be addressed first. The third possibility, that both results are inaccurate, will not be discussed.

It has been extensively demonstrated that analytes in spiked samples are more easily extracted than in incurred samples, and EPA requires total extractability testing using radioactively-labeled pesticides for methods used in pesticide registration. However, the multiresidue methods were not tested for all pesticides in this manner because registrants usually develop single residue methods, thus multiresidue methods were validated using spiked samples. In incurred samples, bound residues may occur that are not extracted by traditional methods [145]. Khan's group has performed several studies using SFE to demonstrate this possibility [123,146–148]. In their study, Aharonson et al. showed that performing the traditional extraction a second time on the pulp of previously extracted samples found more residues. They indicate that the SFE results were higher than a traditional approach due to more complete extraction with the SFE approach for incurred residues.

An alternate explanation is that the subsampling procedure for SFE was not representative of the larger sample. Young et al. show that measured pesticides concentrations tend to increase for a large sample as the subsample size decreases [83]. This is presumably due to the increased effect of moisture losses during the determination of sample weight; a small amount of evaporation can have a significant effect on a small sample. Fig. 4 shows the results for repeated determinations of pesticides with SFE of 2:2:1 celery–$MgSO_4$–HMX. As the original 100 g

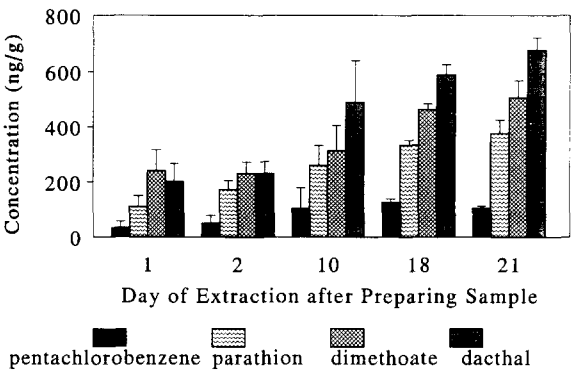


Fig. 4. Results from repeated SFE and GC–ITD analyses of a celery sample (2:2:1 sample–$MgSO_4$–HMX) over the course of several days. The probable cause of the increasing results was loss of moisture.

sample was repeatedly removed from the freezer and subsamples were taken, and evaporation of water caused increasing concentration of the residues in the sample. Careful monitoring of sample weight or the use of a surrogate standard may correct for this concern. Hemingway et al. discuss the use of a surrogate standard to correct for sample weight losses [84]; the surrogate must be homogeneously dispersed in the sample for this approach to work. Reduced loss of water is one other benefit of maintaining a frozen sample conditions throughout the pre-extraction process. If the sample weight is not correct, the subsampling procedure will appear to be inaccurate, even though the pesticides are distributed evenly in the sample.

As in many SFE applications, King et al. were the first to develop multiresidue methods for pesticides in grains [134]. They performed SFE optimization studies for dimethoate, methyl parathion, pirimiphos-methyl, chlorpyrifos, malathion, dieldrin, methoxychlor, and carbofuran. Others have followed with other methods for grains that modify and expand the capabilities of SFE in this application. In studies using an Isco Model 2-10, Ohlin showed excellent agreement between SFE and 2 solvent-based extraction procedures (EtOAc and acetone) for 20 OP and OC pesticides in flour, millet, rye, wheat, dried spinach, and rice [135]. Unlike with the traditional methods, SFE extracts required no further treatment before analysis, and GC–FPD and ECD chromatograms appeared free of interferants. Norden de-

veloped an automated SFE method that has been implemented in the Pesticide Data Program using an Isco Model SFX 3560 for monitoring of diverse pesticides in wheat. His group, in the USDA Federal Grain Inspection Service, makes a modifier addition of 2 ml 80:20 MeCN–water to the sample (which is stored at −80°C) to aid in the extraction of polar pesticides, such as omethoate, thiabendazole, and carbamates (collection in MeCN). This SFE method calls for SPE clean-up with an aminopropyl cartridge before GC–MS and HPLC–fluorescence analyses. Other SFE reports of fewer, yet pertinent, pesticides in grains have appeared [149–151]. Many grains are an uncomplicated matrix for sample preparation due to their low moisture content, good consistency, and minimal matrix interactions. However, soybeans, which have greater lipid content, have been demonstrated to be more problematic in SFE [152].

6. SFE of insecticides

Insecticides consist of 4 major families: organochlorine, N-methylcarbamate, organophosphate, and pyrethroid. The usage of insecticides is widespread in agricultural and other applications, and trace levels of insecticides are often found all types of food and environmental samples. Multiresidue methods often solely address insecticides, which in general, have properties conducive for easy extraction and analysis. The separation of lipids from lipophilic insecticides has been the single greatest concern in their determination.

In meat and similar fatty matrices, King et al. have performed extensive development of SFE method for organochlorine insecticides [107,132,144,154–157]. The separation of co-extracted fats in SFE poses some difficulties, but unique and variable methods of clean-up have been developed for meat and liver [92,125,133,154,157], mussel [159], egg [160,164], breast tissue [161], and similar matrices [139,162,163]. These approaches can be divided into categories involving (1) selective extraction, (2) in-line clean-up, and (3) post-SFE clean-up.

In the first category, a low CO_2 density can be used to gain a degree of selectivity to minimize lipid co-extractives while obtaining incomplete extraction of nonpolar pesticides. However, sacrifice of recovery (<70%) to achieve higher selectivity is typically unacceptable in analytical protocols. Also, even if, for example, 0.1% of fat is extracted from a sample in SFE, this can still pose analytical problems if the sample contains high fat content. Another approach is to use a more polar supercritical fluid that can more selectively extract pesticides and not lipids. Fluoroform has been demonstrated to achieve this effect [157,158], however, inherent drawbacks of prohibitive cost and potential environmental concerns make its routine use currently impractical. In a similar concept, but a much different approach for fatty foods, Argauer et al. avoided much of the lipid co-extractives in SFE by pre-extracting meat samples with MeCN (1:2 meat–MeCN) and partitioning with hexane before performing SFE [92]. This approach works well for relatively polar pesticides, such as carbamates, that do not partition with the fats into the hexane. For lipophilic pesticides, which are more commonly found in meat samples, a MeCN–hexane pesticide partitioning ratio must be factored to obtain accurate results. Again, regulatory requirements do not widely allow this procedure.

In-line clean-up to separate pesticides from fats in tissue matrices has been shown to be very selective. First developed by France et al. this technique involves placing a sorbent between the sample and restrictor [132]. Depending on the sorbent, SFE conditions, and analytes, either the pesticides are retained by the sorbent, or the lipids are retained. For convenience, it is advantageous for the lipids to be retained and for the pesticides to pass for analysis. Murugaverl et al. accomplished this using a 100 mg sorbent column of 7:93 diol–C_{18} for carbamate insecticides in beef and chicken. The use of alumina, Florisil, and silica works similarly for nonpolar pesticides, but these sorbents tend to retain more polar pesticides, which requires that the pesticides be eluted from the sorbent, often in manual fashion using liquids. Other disadvantages of the approach are that: (1) sample size is reduced due to the volume used in the vessel by the sorbent, (2) cost of analysis increases, especially in the case of more selective chromatographic stationary phases, and (3) the approach is less useful for a wide range of pesticides. Hopper has investigated C_1 stationary phase in an attempt to clean-up multiple pesticide classes from co-extracted lipids in SFE with mixed

results [139]. Comparison of in-line versus traditional off-line clean-up can be made for SFE of OC insecticides from eggs [160,164].

Post-SFE clean-up methods include traditional clean-up approaches, and modification of those approaches using supercritical fluids. For example, Nam and King designed an SFE–SFC–GC instrument for extraction–clean-up–analysis of pesticide residue from meat [154], and Stalling et al. designed an SFE–GPC instrument [162]. Taylor et al. took the SFE–GPC concept a step further by using supercritical CO_2 as a mobile phase component [163]. The key to the success of this approach was the use of a densely packed GPC column that could withstand the high pressure of SFE. Separation of pesticides sorted by different classes from lipids was demonstrated with different solvent-CO_2 ratios.

A final way to avoid problems with lipid co-extractives in SFE is to use a method of analysis that is not affected by the presence of fat. ELISA is potentially one such approach (depending on the cross-reactivity of co-extractives with the antibody). Several commercial kits are available for pesticides, and King and Nam reviewed the combination of SFE with ELISA [58]. ELISA and other methods of immunochemical analysis can be used in conjunction with SFE in rapid screening applications. In two such examples, France and King, and Nam and King, developed a unique, inexpensive approach to performing SFE without a gas cylinder or pump [155,156]. They packed a vessel with sample and dry ice, then heated the sealed vessel to moderate temperatures. The pressure of the CO_2 in the vessel exceeded the critical point and after a 30–60 min static extraction, a valve was open to release the fluid. Pressure, thus CO_2 density, of this procedure is limited to $\approx$100 atm, but recoveries of carbofuran, carbendazim, alachlor, atrazine, and 2,4-D were 100% in screening applications of fortified meats. Trapped analytes were analyzed using ELISA after a short filtration step [155].

In the first paper to determine the natural insecticide, abamectin, using SFE, Brooks and Uden developed a method for soil and animal samples [165]. Abamectin consists of avermectins B_{1a} ($C_{48}H_{72}O_{14}$) and B_{1b} ($C_{47}H_{70}O_{14}$), which are macrocyclic lactones. The authors use methoxyethanol modifier to achieve high recoveries of fortified samples (MeOH or MeCN gave low recoveries), but aged fortified soil and meat samples showed 30–50% recoveries. These results could be due to microbial degradation or stronger matrix interactions. In another application involving a natural pesticide, Brooks et al. also developed an SFE method for azadirachtin ($C_{35}H_{44}O_{16}$) for its determination in soil and insects [166]. In this case, MeOH was an effective modifier.

In an SFE application to extract sulfur-containing carbamate insecticides (methomyl, methiocarb, and eptam) from apple, Howard et al. determined that solid-phase trapping was a problem, especially for eptam [167]. A liquid trap was placed after the solid-phase trap in an attempt to improve recovery of eptam, but this approach only achieved moderate success. Norden achieved 100% recoveries of these and other carbamates in wheat using SFE with liquid trapping [136]. Liu et al. achieved high SFE recoveries of the carbamates, isoprocarb, bendiocarb, carbofuran, primicarb, and carbaryl, in apple using a stainless steel trap at 25°C [153].

In the first published combination of SFE with capillary electrophoresis (CE) analysis, Lanças et al. used a homebuilt SFE instrument to extract carbofuran and carbaryl from tobacco [168]. These carbamates can be analyzed using GC, HPLC, or ELISA methods, as well. SFE conditions were 100 atm and 60°C (density=0.4 g/ml) for 2 min, which achieved very low recoveries. The use of 20% acetone modifier was required to achieve high recoveries at these conditions. Clean-up with Florisil was necessary before CE analysis.

7. SFE of fungicides

Several fungicides are used in applications to reduce spoilage of produce and extend shelf-life. Because fungicides are most often applied late in the growing season or after harvest, they are the most commonly detected pesticides in many fruits and vegetables [141]. For the same reason, (benz)imidazoles, triazoles, phthalimides, and similar classes of fungicides, are not typically of concern in environmental samples. In food applications, these types of fungicides often pose difficulties in multiresidue

applications using GC analysis, but GC-based screening methods often include these fungicides for convenience [73–79]. HPLC provides better peak shapes for these fungicides, and for the most reproducibly accurate results, HPLC should be used for their analysis [73,79,81].

In general, SFE has difficulties in extraction of thermally labile chemicals that cannot be analyzed by GC methods [26]. The extraction of relatively polar compounds containing amine, amide, imide, and similar structures that are easily charged, can pose difficulties in SFE. For example, Valverde et al. had difficulty in extraction of imidacloprid [111]. Oostdyk et al. resorted to the use of N_2O as the supercritical fluid for extraction of amine compounds [109]. Nishikawa offers an explanation of this effect for the triazole fungicide, diniconazole, and insecticides, fenitrothion and esfenvalerate, in soil and crop matrices [169]. Hydrogen bonding of pesticides on filter paper and crop matrices in the presence of water was weaker than adsorption interactions of the pesticides on soil. This may explain the reason that SFE applications involving food residues has not demonstrated the problems associated with the use of SFE in environmental analysis [38–42].

Aharonson et al. developed a method for the benzimidazole fungicides, thiabendazole, carbendazim, and thiophanate-methyl, in apple, banana, and potato [115]. HPLC was used for analysis, and in subsequent studies, CE was shown to give better separation and lower detection limits [170]. Jiménez et al. developed a method for carbendazim in lettuce [171]. In the former approach, the samples were mixed with drying agent, and in the latter, samples were lyophilized followed by addition of MeOH modifier. These type of fungicides do not volatilize during lyophilization, but MeOH was shown to have very little effect on recoveries in the presence of water [115].

Using the same homebuilt SFE instrument as in their studies with carbamates [168], Lanças et al. extracted chlorothalonil (tetrachloroisophthalonitrile) from apple [172]. Again, SFE conditions were mild (150 atm and 70°C to give a CO_2 density of 0.52 g/ml) and rapid (3 min), but no modifier was needed in this case to achieve high recovery of chlorothalonil. This fungicide poses degradation problems in multiresidue SFE methods.

Not all fungicides are relatively polar, and Lehotay and Ibrahim developed an SFE method using a Suprex Prepmaster for the organochlorine fungicide, quintozene (pentachloronitrobenzene), and its co-formulants and metabolites, pentachlorobenzene, hexachlorobenzene, pentachloroanisole, pentachlorothioanisole, and pentachloroaniline, in vegetables [98]. This combination of SFE and GC–ITD analysis, which, due to advantages associated with each instrument over traditional methods, provides extraction and detection methods that are well-suited for each other. An alumina trap was demonstrated to adequately separate the analytes from chlorophyll and other potential matrix components extracted by SFE.

8. SFE of herbicides

Herbicides can be divided into many categories based on their usage, properties, and chemical classes. The most heavily applied herbicides are pre-emergent, non-selective herbicides, which are used to clear fields before planting, such as triazines, acetamides, and contact herbicides such as glyphosate and paraquat [70]. Selective herbicides, such as sulfonyl ureas, imidazolinones, and phenoxy acids are used during the growing season to control weeds. Herbicides are rarely found in foods, with the possible exception of the nonpolar dinitroanilines, [141] due to (1) their early-season usage or low usage rates near harvest, (2) typically high solubilities in water, (3) rapid degradation rates, and (4) difficulties in their analysis. With the exception of nonpolar dinitroaniline herbicides, class-specific methods are commonly required for herbicides.

The pre-emergent triazine and acetamide herbicides are the most heavily used pesticides [70], and many SFE papers concerning triazine extraction from soil in particular have been published [173–181]. In foods, the parent triazine and acetamide compounds can be included in multiresidue analysis with GC detection, or ELISA, but metabolites typically unsuitable for GC analysis, or low cross-reactivities in ELISA, are more pertinent for monitoring.

SFE research concerning the extraction of the tetrazine herbicide, metribuzin has also appeared in the literature [148,182]. Dupont and Khan compared

SFE using supercritical acetone with high temperature distillation techniques for bound ^{14}C-labeled metribuzin in soybean plants. Supercritical acetone was achieved at 150 bar and 250°C, and flow through the 1–2 g sample was 1 ml/min for 3 h. This approach found higher ^{14}C concentrations in the samples than high-temperature distillation.

Several publications that have concerned SFE studies involving chlorophenoxy acid herbicides in various matrices have also appeared [183–188]. The use of ion-pairing or derivatization agents prior or during SFE was found to aid the extraction of 2,4-D and similar herbicides [184–187]. Another advantage of this approach was the ability to use GC analysis of the derivatized analytes. Thomson and Chesney compared SFE with steam distillation extraction of 2,4-dichlorophenol in straw and grains [188]. They determined that the use of an acid pretreatment step to the sample prior to SFE increased extracted amounts equal to those found by steam distillation.

Wigfield and Lanouette developed an SFE method for the phenoxy herbicide, fluazifop-*p*-butyl and fluazifop, in onion [189]. Optimized conditions for a Suprex MPS-225 were: 1 ml MeOH modifier added to 1 g freeze-dried sample in a 5 ml vessel, 400 atm at 80°C (0.82 g/ml CO_2 density), 10 min static time and 60 min dynamic time. Lançcas et al. used their homebuilt SFE instrument to extract the pyridazinone herbicide, norflurazon, from cotton seeds [190]. SFE conditions were 100 atm, 70°C (0.26 g/ml CO_2 density) and 5 min dynamic extraction.

Wheeler and McNally investigated SFE and SFC to extract and analyze ^{14}C-labeled phenylurea herbicides, linuron and diuron, in soil [191], and in a later study, McNally et al. extracted the phenylurea herbicide, neburon [192]. Berger developed SFC conditions for separation of phenylureas, which is pertinent in SFE studies because he reports the degradation of these herbicides in supercritical CO_2 at moderate temperatures [193].

In studies of sulfonyl urea herbicides, Howard and Taylor reported conditions for extraction of chlorsulfuron, metsulfuron-methyl, chlorimuron-ethyl, thifensulfuron-methyl, sulfometuron-methyl, tribenuron-methyl, and bensulfuron-methyl from celite, filter paper, and cotton swabs. [194]. Celite and filter paper was observed to retain these herbicides to a larger extent than cotton extent, probably due to the greater surface area of those materials. McNally and Wheeler used SFE–SFE for ^{14}C-labeled sulfonyl urea herbicides in soil and plant samples [195]. No recovery data was presented, but examples showing their extraction, separation, and detection were given. In a total extractability study for a ^{14}C-labeled sulfonyl urea, chloransulam-methyl, in soil, Krieger et al. concluded that SFE with CO_2 was unable to meet the needs for this application [196]. They showed that water as an extractant gave 7-fold higher recoveries (75%) than supercritical CO_2 at nearly maximum density (0.97 g/ml) for the commercial instrument used. Higher recovery was achieved at maximum temperature and pressure, and through the use of modifiers, but recoveries <60% were achieved. Others have also concluded that SFE using supercritical CO_2 is not acceptable for these and other types of polar herbicides in real samples [152,197]. These types of herbicides require extraction using water-based systems with careful control of pH.

Only one report on the use of SFE for the extraction of imidazolinone herbicides (imazaquin from soil) has appeared [198]. Supercritical CO_2 is ineffective to extract these types of pesticides, but this feature can possibly be used to the analyst's advantage if matrix interferants are extracted while the analytes remain in the vessel. A different solvent can then be used to extract the analyte free of the interferant. This approach, which was labeled, 'inverse SFE' [199], may prove very useful in complementary approaches with liquid-based extraction.

No studies involving SFE has been published pertaining to the extraction of thiocarbamate herbicides in pesticide residue applications, but thiocarbamates have been extracted from diverse samples using SFE. Thiocarbamates are used as chelating agents to aid in the SFE of metal ions [55–57]. There is no apparent reason that these approaches cannot be used to extract thiocarbamate residues from applicable food and environmental samples.

9. Conclusions

Regulatory laboratories, the food industry, and

independent testing laboratories around the world are likely to perform 100 000–200 000 analyses per year to monitor pesticide residues in food [200]. Many current methods of analysis used for the regulation of pesticides in foods are estimated to release 10^8 times more potentially hazardous chemicals into the environment than the level of residues in the sample [201]. These types of methods also burden the testing laboratories with high costs, a great deal of manual labor, and large space requirements. Due to external pressures placed on routine laboratories, there is a growing need to implement more environmentally and fiscally sound methods [202].

With the use of sample size reduction and advanced analytical instrumentation, classical methods of extraction can be modified to reduce costs and increase convenience of existing methods, but these modified methods can still not match the ease and increased selectivity of SFE. A significant inherent advantage in the use of SFE versus solvent-based extraction methods is the concentration of the analytes during the collection process in SFE.

This article has discussed the use of SFE in pesticide residue analysis with the expectation that chemists in routine laboratories will begin to investigate and implement SFE methods in their laboratory applications. As commercial SFE instruments improve, and more analysts become familiar with SFE techniques, new SFE approaches will be developed to meet other specific needs in pesticide analysis.

Limitations will always remain in the range of pesticides that can be extracted by SFE with a single extraction fluid. The use of water as an environmentally friendly solvent for extraction of polar pesticides followed by concentration using solid-phase cartridges serves as a convenient, inexpensive complementary method to SFE. In the foreseeable future, automated instruments will be able to use supercritical and liquid solvents interchangeably that will allow extraction of virtually any pesticide from any matrix. Boundaries in the range of pesticides that are currently set by classical analytical schemes can be redrawn to maximize the advantages of SFE and water-based extraction. Emerging methods of pesticide analysis, using such instruments as GC–MS–MS, GC–AED, HPLC–MS, capillary electrophoresis, and immunochemical techniques, may be incorporated into an overall approach using SFE and water for extraction.

Acknowledgements

The author is grateful to Robert Epstein and the support of the Pesticide Data Program (USDA Agricultural Marketing Service), and to Nadav Aharonson, Konstantin Eller, Antonio Valverde García and Satoru Nemoto. The author also thanks Hewlett-Packard and Suprex for the loan of SFE instruments.

References

[1] L.T. Taylor, Supercritical fluid extraction, Wiley, New York, NY, 1996.
[2] G. Brunner, Gas extraction: An introduction to fundamentals of supercritical fluids and the application to separation processes, Steinkopff, Darmstadt, Germany, 1994.
[3] J.R. Dean (Ed.), Applications of supercritical fluids in industrial analysis, Blackie, New York, NY, 1993.
[4] M.A. McHugh, V.J. Krukonis, Supercritical fluid extraction: Principles and practice, 2nd ed., Butterworth-Heinemann, Boston, MA, 1994.
[5] M.A. McHugh, V.J. Krukonis, Supercritical fluid extraction: Principles and practice, Butterworths, Boston, MA, 1986.
[6] B. Wenclawiak (Ed.), Analysis with supercritical fluids: Extraction and chromatography, Springer-Verlag, New York, NY, 1992.
[7] S.A. Westwood (Ed.), Supercritical fluid extraction and its use in chromatographic sample preparation, Blackie, Glasgow, UK, 1992.
[8] M. Saito, Y. Yamauchi, T. Okuyama (Eds.), Fractionation packed-column SFC SFE, VCH, New York, NY, 1994.
[9] K. Jinno (Ed.), Hyphenated techniques in supercritical fluid chromatography and extraction, J. Chromatogr. Library 53 (1992).
[10] T.J. Bruno, J.F. Ely (Eds.), Supercritical fluid technology: Reviews in modern theory and applications, CRC Press, Boca Raton, FL, 1991.
[11] S.S.H. Rizvi (Ed.), Supercritical fluid processes for food biomaterials, Blackie, Glasgow, UK, 1994.
[12] E. Kiran, J.F. Brennecke (Eds.), Supercritical engineering science: Fundamentals and applications, ACS Symposium Series 514, American Chemical Society, Washington, DC, 1993.
[13] K.W. Hutchenson, N.R. Foster (Eds.), Innovations in supercritical fluids, ACS Symposium Series 608, American Chemical Society, Washington, DC, 1995.
[14] F.V. Bright, M.E.P. McNally (Eds.), Supercritical fluid technology: Theoretical and applied approaches to analytical chemistry, ACS Symposium Series 488, American Chemical Society, Washington, DC, 1992.
[15] K.P. Johnson, J.M.L. Penninger (Eds.), Supercritical fluid science and technology, ACS Symposium Series 406, American Chemical Society, Washington, DC, 1989.

[16] B.A. Carpenter, M.R. Sevanants (Eds.), Supercritical fluid extraction and chromatography: Techniques and applications, ACS Symposium Series 366, American Chemical Society, Washington, DC, 1988.
[17] M.L. Lee, K.E. Markides (Eds.), Analytical supercritical fluid chromatography and extraction, Chromatography Conferences, Provo, UT, 1990.
[18] T.L. Chester, J.D. Pinkston, D.E. Raynie, Anal. Chem. 68 (1996) 487R–514R.
[19] T.L. Chester, J.D. Pinkston, D.E. Raynie, Anal. Chem. 66 (1994) 106R–130R.
[20] T.L. Chester, J.D. Pinkston, D.E. Raynie, Anal. Chem. 64 (1992) 153R–170R.
[21] T. Clifford, K. Bartle, Chem. Br. 29 (1993) 499–502.
[22] D.R. Gere, E.M. Derrico, LC-GC 12 (1994) 352–366.
[23] D.R. Gere, E.M. Derrico, LC-GC 12 (1994) 432–441.
[24] J.W. King, J. Chromatogr. Sci. 27 (1989) 355–364.
[25] J.W. King, M.L. Hopper, J. AOAC Int. 75 (1992) 375–378.
[26] S.B. Hawthorne, Anal. Chem. 62 (1990) 633A–642A.
[27] M.E.P. McNally, J. AOAC Int. 79 (1996) 380–387.
[28] J.L. Hedrick, L.J. Mulcahey, L.T. Taylor, Mikrochim. Acta 108 (1992) 115–132.
[29] M.E.P. McNally, Anal. Chem. 67 (1995) 308A–315A.
[30] L.T. Taylor, Anal. Chem. 67 (1995) 364A–370A.
[31] M.E. Paulitis, V.J. Krukonis, R.T. Kurnik, R.C. Reid, Rev. Chem. Engr. 1 (1993) 179–250.
[32] X. Lou, H. Janssen, C.A. Cramers, J. High Resolut. Chromatogr. 18 (1995) 483–489.
[33] J.R. Dean, Analyst 121 (1996) 85R–89R.
[34] A. Akgerman, Waste Manage. 13 (1993) 403–415.
[35] F. Hoefler, Laborpraxas 18 (1994) 60–68.
[36] V. Lopez-Avila, Environ. Test. Anal. 4 (1995) 44–49.
[37] I.J. Barnabas, J.R. Dean, S.P. Owen, Analyst 119 (1994) 2381–2394.
[38] S. Bøwadt, S.B. Hawthorne, J. Chromatogr. A 703 (1995) 549–571.
[39] J.M. Levy, A.C. Rosselli, in: G. Subramanian (Ed.), Quality assurance in environmental monitoring, VCH, Weinheim, Germany, 1995, pp. 25–54.
[40] V. Janda, K.D. Bartle, A.A. Clifford, J. Chromatogr. 642 (1993) 283–299.
[41] T.S. Reighard, S.V. Olesik, Crit Rev. Anal. Chem. 26 (1996) 61–99.
[42] J.R. Dean, J. Chromatogr. A 754 (1996) 221–233.
[43] J.M. Levy, V. Danielson, R. Ravey, L. Dolata, LC-GC 12 (1994) 920–923.
[44] K.D. Bartle, A.A. Clifford, Spec. Publ. R. Soc. Chem. 160 (1994) 1–41.
[45] K.D. Bartle, T.A. Clifford, Adv. Appl. Lipid Res. 1 (1992) 217–264.
[46] T. Paschke, Laborpraxis 19 (1995) 24–26.
[47] F. Kelnhofer, G. Tittel, Int. Lab. 13 (1993) 16–21.
[48] C.D. Bevan, P.S. Marshall, Nat. Prod. Rep. 11 (1994) 451–466.
[49] R.M. Smith, M.D. Burford, Sample Prep. Biomed. Environ. Anal. 1 (1994) 3–19.
[50] R.M. Smith, LC-GC 13 (1995) 930–939.
[51] W.K. Modey, D.A. Mulholland, M.W. Raynor, Phytochem. Anal. 7 (1996) 1–15.
[52] J.M. Bruna, Rev. Plast. Mod. 69 (1995) 448–451.
[53] J. Via, L.T. Taylor, Chemtech (1993) 38–44.
[54] J.W. King, Trends Anal. Chem. 14 (1995) 474–481.
[55] Y. Lin, N.G. Smart, C.M. Wai, Trends Anal. Chem. 14 (1995) 123–132.
[56] N. Smart, Y. Lin, C.M. Wai, Am. Environ. Lab. 8 (1996) 38–42.
[57] M.D. Luque de Castro, M.T. Tena, Trends Anal. Chem. 15 (1996) 32–37.
[58] J.W. King, K.-S. Nam, in: R.C. Beier, L.H. Stanker (Eds.), Immunoassays for residue analysis, ACS Symposium Series 621, American Chemical Society, Washington, DC, 1996, pp. 422–438.
[59] C. Staub, P. Edder, J.-L. Veuthey, in: P. Kintz (Ed.), Drug testing of hair, CRC Press, Boca Raton, Florida, 1996, pp. 121–149.
[60] T. Greibrokk, J. Chromatogr. A 703 (1995) 523–536.
[61] V. Janda, M. Mikešová, J. Vejrosta, J. Chromatogr. A 733 (1996) 35–40.
[62] F. Wach, Anal. Chem. 66 (1994) 369A–373A.
[63] B.E. Richter, in: T. Cairns, J. Sherma (Eds.), Emerging strategies for pesticide analysis, CRC Press, Boca Raton, Florida, 1992, pp. 51–68.
[64] I.A. Stuart, J. MacLachlin, A. McNaughtan, Analyst 121 (1996) 11R–28R.
[65] The pesticide manual, British Crop Protection Council, Surrey, UK.
[66] Herbicide Handbook of the Weed Science Society of America, Weed Science Society of America, Champaign, IL.
[67] Farm chemicals handbook '96, Meister, Willoughby, OH, 1996.
[68] Farm chemicals handbook electronic pesticide dictionary '96, Meister, Willoughby, OH, 1996.
[69] A.G. Hornsby, R.D. Wauchope, A.E. Herner, Pesticide properties in the environment, Springer-Verlag, New York, NY, 1996.
[70] Agricultural chemical usage 1995 field crops summary, USDA National Agricultural Statistics Service, Washington, DC, 1996.
[71] S.J. Lehotay, C.-H. Lee, J. Chromatogr. A, 785 (1997) in press.
[72] C.H. Parfitt, Jr., Laboratory Information Bulletin, US Food and Drug Administration, Vol. 7, No. 3616, 1991.
[73] Pesticide analytical manual, Vol. I, Multiresidue methods, 3rd ed., US Food and Drug Administration, Washington, DC, 1994.
[74] M.A. Luke, H.T. Masumoto, Anal. Meth. Pest. Plant Growth Reg. 15 (1986) 161–200.
[75] T. Cairns, M.A. Luke, K.S. Chiu, D. Navarro, E.G. Siegmund, Rap. Commun. Mass Spectrom. 7 (1993) 1070–1076.
[76] S.M. Lee, M.L. Papathakis, H.M.C. Feng, G.F. Hunter, J.E. Carr, Fresenius J. Anal. Chem. 339 (1991) 376–383.
[77] J. Fillion, R. Hindle, M. Lacroix, J. Selwyn, J. AOAC Int. 78 (1995) 1252–1266.
[78] A. Andersson, H. Pålsheden, Fresenius J. Anal. Chem. 339 (1991) 365–367.
[79] Analytical methods for pesticide residues in foodstuffs, 6th ed., General Inspectorate for Health Protection, The Netherlands, 1996.

[80] A. de Kok, M. Hiemstra, J. AOAC Int. 75 (1992) 1063–1072.
[81] M. Hiemstra, J.A. Joosten, A. de Kok, J. AOAC Int. 78 (1995) 1267–1274.
[82] D.R. Erney, A.M. Gillespie, D.M. Gilvydis, C.F. Poole, J. Chromatogr. 638 (1993) 57–63.
[83] S.V. Young, C.H. Parfitt Jr., R.F. Newell, T.D. Spittler, J. AOAC Int. 79 (1996) 976–980.
[84] R.J. Hemingway, N. Aharonson, P.A. Greve, T.R. Roberts, H.P. Thier, Pure and Appl. Chem. 56 (1984) 1131–1152.
[85] A. Di Muccio, S. Girolimetti, D. Attard Barbini, T. Generali, P. Pelosi, A. Ausili, F. Vergori, G. De Merulis, A. Santillo, I. Camoni, 1st European Pesticide Residue Workshop, Alkmaar (Egmond aan Zee), The Netherlands, June 10–12, 1996, abstract P-118.
[86] S.J. Lehotay, N. Aharonson, E. Pfeil, M.A. Ibrahim, J. AOAC Int. 78 (1995) 831–840.
[87] K.D. Parker, M.L. Dewey, D.M. DeVries, S.C. Lau, Toxicol. Appl. Pharmacol. 7 (1965) 719.
[88] J.E. Benville Jr., R.C. Tindle, J. Agric. Food Chem. 18 (1970) 948–949.
[89] E.A. Bunch, D.M. Altwein, L.E. Johnson, J.R. Farley, A.A. Hammersmith, J. AOAC Int. 78 (1995) 883–887.
[90] J. Nelson, H. Abdelmesseh, Laboratory information bulletin, US Food and Drug Administration, Vol. 7, No. 3560, 1991.
[91] L.J. Mulcahey, L.T. Taylor, Anal. Chem. 64 (1992) 2352–2358.
[92] R.J. Argauer, K.I. Eller, M.A. Ibrahim, R.T. Brown, J. Agric. Food Chem. 43 (1995) 2774–2778.
[93] S. Bengtsson, T. Berglöf, S. Granat, G. Jonsäll, Pestic. Sci. 41 (1994) 55–60.
[94] M.F. Wolfe, D.E. Hinton, J.N. Seiber, Environ. Toxicol. Chem. 14 (1995) 1001–1009.
[95] V. Lopez-Avila, N.S. Dodhiwala, W.F. Beckert, J. Chromatogr. Sci. 28 (1990) 468–476.
[96] P.R. McEachern, G.D. Foster, J. Chromatogr. 632 (1993) 119–125.
[97] R. Alzaga, G. Durand, D. Barceló, J.M. Bayona, Chromatographia 38 (1994) 502–507.
[98] S.J. Lehotay, M.A. Ibrahim, J. AOAC Int. 78 (1995) 445–452.
[99] J.W. King, J.H. Johnson, S.L. Taylor, W.L. Orton, M.L. Hopper, J. Supercrit. Fluids 8 (1995) 167–175.
[100] M.L. Hopper, J.W. King, J.H. Johnson, A.A. Serino, R.J. Butler, J. AOAC Int. 78 (1995) 1072–1079.
[101] T.M. Fahmy, M.E. Paulaitis, D.M. Johnson, M.E.P. McNally, Anal. Chem. 65 (1993) 1462–1469.
[102] M.S. Kuk, J.C. Montagna, in: M.E. Paulaitis, J.M. Penninger, R.D. Gray, K.P. Davison (Eds.), Chemical engineering at supercritical conditions, Ann Arbor Science, Ann Arbor, MI, 1983, pp 101–111.
[103] S. Nemoto, K. Sasaki, M. Toyoda, Y. Saito, J. Chromatogr. Sci., in press.
[104] J.L. Snyder, R.L. Grob, M.E. McNally, T.S. Oostdyk, J. Chromatogr. Sci. 31 (1993) 183–191.
[105] J.L. Snyder, R.L. Grob, M.E. McNally, T.S. Oostdyk, J. Environ. Sci. Health A29 (1994) 1801–1816.
[106] M.D. Burford, S.B. Hawthorne, D.J. Miller, J. Chromatogr. A 657 (1993) 413–427.
[107] M.L. Hopper, J.W. King, J. Assoc. Off. Anal. Chem. 74 (1991) 661–666.
[108] S.J. Lehotay, K.I. Eller, J. AOAC Int. 78 (1995) 821–830.
[109] T.S. Oostdyk, R.L. Grob, J.L. Snyder, M.E.P. McNally, J. Chromatogr. Sci. 31 (1993) 177–182.
[110] A. Valverde-García, A.R. Fernández-Alba, A. Aguera, M. Contreras, J. AOAC Int. 78 (1995) 867–873.
[111] A. Valverde-García, A.R. Fernandez-Alba, M. Contreras, A. Agüera, J. Agric. Food Chem. 44 (1996) 1780–1784.
[112] K.I. Eller and S.J. Lehotay, Analyst, 122 (1997) 429–436.
[113] R. Stefani, M. Buzzi, R. Grazzi, 1st European Pesticide Residue Workshop, Alkmaar (Egmond aan Zee), The Netherlands, June 10–12, 1996, abstract P-033.
[114] M.L. Hopper, pers. comm.
[115] N. Aharonson, S.J. Lehotay, M.A. Ibrahim, J. Agric. Food Chem. 42 (1994) 2817–2823.
[116] M. Ashraf-Khorassani, L.T. Taylor, Am. Labor., December, 1995, pp. 23–28.
[117] J.M. Levy, L. Dolota, R.M. Ravey, E. Storozynsky, K.A. Holowczak, J. High Resol. Chromatogr. 16 (1993) 368–371.
[118] K.M.S. Sundaram, R. Nott, Environ. Sci. Health B30 (1995) 175–200.
[119] B.E. Richter, B.A. Jones, J.L. Ezzell, N.L. Porter, N. Avdalovic, C. Pohl, Anal. Chem. 68 (1996) 1033–1039.
[120] H. Obana, K. Kikuchi, M. Okahishi, S. Hori, Analyst 122 (1997) 217–220.
[121] V. Lopez-Avila, R. Young, N. Teplitsky, J. AOAC Int. 79 (1996) 142–156.
[122] P. Capriel, A. Haisch, S.U. Khan, J. Agric. Food Chem. 34 (1986) 70–73.
[123] J.A. Blackwell, G.O. Cantrell, J.D. Weckworth, P.W. Carr, 7th Int. Symp. Supercrit. Fluid Chromatogr. Extr. Oral and Poster Abstracts, Indianapolis, IN, March 31–April 4, 1996, abstract A-06.
[124] O.W. Parks, R.J. Maxwell, J. Chromatogr. Sci. 32 (1994) 290–293.
[125] N. Din, K.D. Bartle, A.A. Clifford, A. McCormack, L. Castle, J. Chromatogr. Sci. 35 (1997) 31–37.
[126] J.K. Rice, E.D. Niemeyer, F.V. Bright, Anal. Chem. 67 (1995) 4354–4357.
[127] P.R. Eckard, L.T. Taylor, J. High Resol. Chromatogr. 19 (1996) 117–120.
[128] P.G. Thompson, L.T. Taylor, B.E. Richter, N.L. Porter, J.L. Ezzell, J. High Resol. Chromatogr. 16 (1993) 713–716.
[129] L.J. Mulcahey, J.L. Hedrick, L.T. Taylor, Anal. Chem. 63 (1991) 2225–2237.
[130] W.N. Moore, L.T. Taylor, Anal. Chem. 67 (1995) 2030–2036.
[131] S.J. Lehotay, A. Valverde-García, J. Chromatogr. A. 765 (1997) 69–84.
[132] J.E. France, J.W. King, J.M. Snyder, J. Agric. Food Chem. 39 (1991) 1871–1874.
[133] B. Murugaverl, A. Gharaibeh, K.J. Voorhees, J. Chromatogr. A 657 (1993) 223–226.

[134] J.W. King, M.L. Hopper, R.G. Luchtefeld, S.L. Taylor, W.G. Orton, J. AOAC Int. 76 (1993) 857–864.
[135] B. Ohlin, Book of Abstracts, Vol. 1, 8th IUPAC International Congress of Pesticide Chemistry, Washington, DC, July 4–9, 1994, abstract 334.
[136] T. Norden, pers. comm.
[137] K.N.T. Norman, W.A. Matthews, M. Haverly, 8th California Pesticide Residue Workshop, San Francisco, CA, March 10–14, 1996, poster abstract 16.
[138] J.W. King, J.M. Snyder, S.L. Taylor, J.H. Johnson, L.D. Rowe, J. Chromatogr. Sci. 31 (1–5).
[139] M.L. Hopper, J. AOAC Int. 80 (1997) 639–646.
[140] S. Hosoi, H. Kikawa, M. Fujita, T. Maeda, Proceedings from the 5th SFC/E Conference, Baltimore, MD, January 11–14, 1994, abstract F-8.
[141] Pesticide Data Program Annual Summary Calendar Year 1994, USDA Agricultural Marketing Service, Washington, DC, 1996.
[142] N. Aharonson, P. Goldshlag, N. Spravchikov, M. Freund, 1st European Pesticide Residue Workshop, Alkmaar (Egmond aan Zee), The Netherlands, June 10–12, 1996, abstract P-021.
[143] N. Aharonson, pers. comm.
[144] J.M. Snyder, J.W. King, L.D. Rowe, J.A. Woerner, J. AOAC Int. 76 (1993) 888–892.
[145] R. Huber, S. Otto, in: J. Miyamoto, P.C. Kearney (Eds.), Pesticide science and biotechnology, Vol. 3, Pergamon, Oxford, UK, 1983, pp. 357–362.
[146] S.U. Khan, J. Agric. Food Chem. 43 (1995) 1718–1723.
[147] A. Paquet, S.U. Khan, J. Agric. Food Chem. 43 (1995) 843–848.
[148] S. Dupont, S.U. Khan, J. Agric. Food Chem. 40 (1992) 890–893.
[149] Z.V. Skopec, R. Clark, P.M.A. Harvey, R.J. Wells, J. Chromatogr. Sci. 31 (1993) 445–449.
[150] A. Izquierdo, M.T. Tena, M.D. Luque de Castro, M. Valcárcel, Chromatographia 42 (1996) 206–212.
[151] R.M. Campbell, D.M. Meunier, H.J. Cortes, J. Microcolumn Separations 1 (1989) 303–308.
[152] S. Nemoto and S.J. Lehotay, 110th AOAC International Annual Meeting, Orlando, FL, 8–12 September 1996, Abstract No. 14-B-003
[153] Y. Liu, W. Zhang, Y. Qui, Chin. J. Chromatogr. 14 (1996) 457–459.
[154] K.-S. Nam, J.W. King, J. High Resol. Chromatogr. 17 (1994) 577–582.
[155] K.-S. Nam, J.W. King, J. Agric. Food Chem. 42 (1994) 1469–1474.
[156] J.E. France, J.W. King, J. Assoc. Off. Anal. Chem. 74 (1991) 1013–1016.
[157] S.L. Taylor, J.W. King, Air Products applications bulletin, No. 320-9431, Air Products, PA, 1994.
[158] M. Ashraf-Khorassani, L.T. Taylor, J. Agric. Food Chem. 44 (1996) 3540–3547.
[159] W.J. Yoo, L.T. Taylor, B.A. Benner, Jr., M.M. Schantz, S.A. Wise, 7th Int. Symp. Supercrit. Chromatogr. Extr. Oral and Poster Abstracts, Indianapolis, IN, March 31–April 4, 1996, abstract C-09.
[160] W. Fiddler, J.W. Pensabene, R.A. Gates, 110th AOAC International Annual Meeting, Orlando, FL, 8–12 September 1996, Abstract No. 14-C-004.
[161] M.V. Djordjevic, D. Hoffmann, J. Fan, B. Prokopczyk, M.L. Citron, S.D. Stelman, Carcinogenesis 15 (1994) 2581–2585.
[162] D.L. Stalling, S. Saim, K.C. Kuo, J.J. Stunkel, J. Chromatogr. Sci. 30 (1992) 486–490.
[163] S.L. Taylor, J.W. King, F. Favati, M.L. Hopper, 7th Int. Symp. Supercrit. Chromatogr. Extr. Oral and Poster Abstracts, Indianapolis, IN, March 31–April 4, 1996, abstract L-25.
[164] Y.Y. Wigfield, J. Selwyn, S. Khan, R. McDowell, Chemosphere 32 (1996) 841–847.
[165] M.W. Brooks, P.C. Uden, Pestic. Sci. 43 (1995) 141–146.
[166] M.W. Brooks, S. Roy, P.C. Uden, P. Vittum, J. Test. Eval. 24 (1996) 149–153.
[167] A.L. Howard, C. Braue, L.T. Taylor, J. Chromatogr. Sci. 31 (1993) 323–329.
[168] F.M. Lanças, S.R. Rissato, M.S. Galhiane, Chromatographia 42 (1996) 323–328.
[169] Y. Nishikawa, Anal. Sci. 7 (1991) 567–572.
[170] A.J. Krynitsky, S.J. Lehotay, N. Aharonson, unpublished results.
[171] J.J. Jiménez, J. Atienza, J.L. Bernal, L. Toribio, Chromatographia 38 (1994) 395–399.
[172] F.M. Lanças, M.A. Barbirato, M.S. Galhiane, S.R. Rissato, Chromatographia 42 (1996) 547–550.
[173] S. Papilloud, W. Haerdi, S. Chiron, D. Barcelo, Environ. Sci. Technol. 30 (1996) 1822–1826.
[174] S. Papilloud, W. Haerdi, Chromatographia 40 (1995) 705–711.
[175] S. Papilloud, W. Haerdi, Chromatographia 38 (1994) 514–519.
[176] T.R. Steinheimer, R.L. Pfeiffer, K.D. Scoggin, Anal. Chem. 66 (1994) 645–650.
[177] E.G. van der Velde, M.R. Ramlal, A.C. van Beuzekom, R. Hoogerbrugge, J. Chromatogr. A 683 (1994) 125–139.
[178] S. Shah, M. Ashraf-Khorassani, L.T. Taylor, J. Chromatogr. 505 (1990) 293–298.
[179] S.B. Hawthorne, D.J. Miller, Anal. Chem. 66 (1994) 4005–4012.
[180] V. Janda, G. Steenbeke, P. Sandra, J. Chromatogr. 479 (1989) 200–205.
[181] G.K. Stearman, M.J.M. Wells, S.M. Adkisson, T.E. Ridgill, Analyst 120 (1995) 2617–2621.
[182] R.W. Malone, R.C. Warner, M.E. Byers, D.J. Hilborn, D. Gere, 1995 Int. Summer Meeting of Am. Soc. Agric. Eng., Chicago, IL, June 18–23, 1995, paper 952435.
[183] E.A. Rochette, J.B. Harsh, H.H. Hill Jr., Environ. Sci. Technol. 30 (1996) 1220–1226.
[184] V. Lopez-Avila, N.S. Dodhiwala, W.F. Beckert, J. Agric. Food Chem. 41 (1993) 2038–2044.
[185] S.B. Hawthorne, D.J. Miller, D.E. Nivens, D.C. White, Anal. Chem. 64 (1992) 405–412.
[186] R. Hillmann, K. Bachmann, J. High Resol. Chromatogr. 17 (1994) 350–352.

[187] S.N. Chatfield, M.Y. Croft, T. Dang, E.J. Murby, G.Y.F. Yu, R.J. Wells, Anal. Chem. 67 (1995) 945–951.
[188] C.A. Thomson, D.J. Chesney, Anal. Chem. 64 (1992) 848–853.
[189] Y.Y. Wigfield, M. Lanouette, J. Agric. Food Chem. 65 (1993).
[190] F.M. Lanças, M.A. Barbirato, M.S. Galhaine, Chromatographia 40 (1995) 432.
[191] J.R. Wheeler, M.E. McNally, J. Chromatogr. Sci. 27 (1989) 534–539.
[192] M.E.P. McNally, C.M. Deardorf, T.M. Fahmy, in: Supercritical fluid extraction: New directions and understandings, ACS Symposium Series 488, American Chemical Society, Washington, DC 1992, pp. 144–164.
[193] T.A. Berger, J. Chromatogr. Sci. 32 (1994) 25–29.
[194] A.L. Howard, L.T. Taylor, J. High Resol. Chromatogr. 16 (1993) 39–45.
[195] M.E.P. McNally, J.R. Wheeler, J. Chromatogr. 435 (1988) 63–71.
[196] M.S. Kreiger, R.N. Yoder, J.L. Wynn, 7th Int. Symp. Supercrit. Fluid Chromatogr. Extr. Oral and Poster Abstracts, Indianapolis, IN, March 31–April 4, 1996, abstract E-17.
[197] M.M. Safarpour, pers. comm.
[198] K.N. Reddy, M.A. Locke, Weed Sci. 42 (1994) 249–253.
[199] D.C. Messer, L.T. Taylor, Anal. Chem. 66 (1994) 1591–1592.
[200] J.H. Hotchkiss, Crit. Rev. Food Sci. Nutr. 31 (1992) 191–203.
[201] H. Steinwandter, in: T. Cairns, J. Sherma (Eds.), Emerging strategies for pesticide analysis, CRC Press, Boca Raton, FL, 1992, pp. 3–38.
[202] T. Cairns, in: T. Cairns, J. Sherma (Eds.), Emerging strategies for pesticide analysis, CRC Press, Boca Raton, FL, 1992, pp. 331–335.
[203] K.L. Pearce, V.C. Trenerry, S. Were, J. Agric. Food Chem. 45 (1997) 153–157.